MMP13 F 56 S mutation is not associated with Spondyloepimetaphyseal Dysplasia: Handigodu type

Pulamaghatta N. Venugopal¹, Cholendra Arja², Adimoolam Chandrasekar¹, Arjun Rao¹

¹Anthropological Survey of India, Southern Regional Centre, Bogadi, Mysore, Karnataka, 570026, India; E-mail: adimoolamchandrasekar@yahoo.com
²Division of Human Genetics, Department of Anthropology, Sri Venkateshwara University, Tirupati, Andhra Pradesh, 517502, India

ABSTRACT

Handigodu (HG) syndrome is a disorder of the osteoarticular system prevalent in few villages of two districts of the Karnataka state in Southern India. The condition was first observed from a Handigodu village, hence its name. Subsequent multidisciplinary study by the Indian Council of Medical Research (ICMR), Government of India revealed that Handigodu syndrome could be considered as late onset spondylo epiphyseal dysplasia. Genomic DNA was isolated from 5ml of peripheral blood samples of Handigodu syndrome affected individuals and controls further it has been sequenced exon 2 of MMP 13 gene to identify the mutation at the codon F 56 S. The sequence analysis of exon 2 of MMP13 did not reveal any polymorphisms, including the functional F 56 S, in both patients and controls. MMP 13 is important for the replacement of cartilage with bone and not in the elongation of long bones, which may be controlled by its upstream signalling molecules. This explains the absence of MMP 13 mutation in HG syndrome patients and suggests a possibility of defect in the upstream signalling molecules.

Keywords: Handigodu Syndrome, Spondyloepimetaphyseal Dysplasia, MMP13, endochondral ossification

INTRODUCTION

Handigodu (HG) syndrome is a rare and painful osteoarthritic disorder endemic to the Malnad region (Shimoga and Chikkmaglur districts) of Karnataka state, India. The first case of the disease was reported from Handigodu Village of Sagar Taluk of Shimoga District, hence the name of the disease. Despite several studies the condition continues to remain a major medical problem of these socially deprived people. The affected individuals are categorized into 3 phenotypes: type I (predominantly hip-joint pains), type II (predominantly dysplastic), and type III (dwarf). Dysplasia of hip joints and spine is often associated with osteoarthritis. Radiological findings in HG syndrome (Figure 1) include narrowing of the joint space, irregularity and sclerosis of articular margins, presence of osteophytes, protrusio acetabuli, flattening of femoral head, flattening and fragmentation of epiphyses, coxa vara, subchondral sclerosis, small pelvis, broad short neck, wedge-shaped vertebrae, irregularity of end plates, and platyspondyly [1].

Since its discovery in 1975, the etiology of this disease remains unknown till day. Earlier studies conducted by Indian Council of Medical Research and National Institute of Nutrition could not attribute the cause to any ecological disturbances, like trace metal toxicities or undue exposure to pesticides. An earlier study identified this disease as a type of spondyloepimetaphyseal dysplasia (SEMD) which follows a predominantly autosomal dominant pattern of inheritance [1]. However, its pattern of inheritance is not validated [2,3]. The earlier study [4] mapped the locus, which is
responsible for SEMD Missouri type (SEMD$_{MO}$) on chromosome 11 (11q14.3-23.2). Further, the sequence analysis in this region identified a mutation in the exon 2 (F 56 S) in the MMP13 gene, which leads to intracellular autoactivation and degradation of the mutant proenzyme, which is likely to be misfolded, with the resulting MMP13 deficiency causing SEMD$_{MO}$ [4]. As radiological features of SEMD$_{MO}$ are similar to SEMD$_{HG}$, we tested HG syndrome patients for F 56 S mutation in exon 2 of MMP13.

Figure 1. Radiographic abnormalities of SEMD$_{HG}$.

**MATERIALS AND METHODS**

All patients included in the study were those identified by Indian Council of Medical Research on the basis of radiological findings. Information regarding patients was obtained from the Medical officer of HG Syndrome Mobile Health Unit of Shimoga District. About 5ml of peripheral blood was collected from 200 SEMD$_{HG}$ patients and 100 controls after receiving their informed consent. Genomic DNA was isolated using phenol-chloroform method. Amplification of exon 2 of MMP13 was carried out in a 10µl reaction mixture using the forward primer 5'-TGCCAATCCTGATGATGCGGT-3' and reverse primer 5'-GTGGAACTCTTCATCTTGAGCACT-3'. The reactions were performed with an initial denaturing cycle of 5 minutes at 95°C, followed by 35 cycles at 94°C for 1 minute, 63.8°C for 45 seconds, 72°C for 2 minutes 30 seconds, and a final extension for 7 minutes at 72°C. The polymerase chain reaction product was confirmed on a 2% w/v agarose gel. Polymerase chain reaction products were sequenced using Applied Bio systems ABI 3730 sequencer.

**RESULTS AND DISCUSSION**

The sequence analysis of exon 2 of MMP13 did not reveal any polymorphisms, including the functional F 56 S, in both patients and controls (Figure 2). Fetal bone development is a complex
process, which occurs by means of intramembraneous ossification and endochondral ossification. The relevance of MMP13 in SEMD comes from its indispensable role in endochondral ossification in which long bone-like limbs are formed from cartilage. Periarticular chondrocytes first proliferate and differentiate into columnar chondrocytes. Columnar chondrocytes determine the length of the bone. The columnar chondrocytes proliferate and differentiate into prehypertrophic chondrocytes, which in turn differentiate into hypertrophic chondrocytes.

Figure 2. Electrophorograph of the position of 56 codon.

Growth plate lies between the two centres of ossification. Formation of bone proceeds toward the plate from both the directions, but the growth of cartilage is faster on one side (diaphysis) which allows for elongation. During endochondral ossification, the mitotically active proliferating chondrocytes produce an extracellular matrix composed principally of collagen type II. As chondrocytes differentiate (postmitotic), they synthesize collagen X [5]. The most mature hypertrophic chondrocytes produce alkaline phosphatase which deposits calcium in the cartilaginous matrix. The hypertrophic chondrocytes undergo apoptosis as nutrients can no longer diffuse through the calcified matrix. This creates cavities in the bone. The hypertrophic chondrocytes also express vascular endothelial growth factor, which allows the corresponding area to be invaded by blood vessels which brings into the cavity hematopoietic cells, osteoclast, and osteoblasts. The osteoblasts then produce osteoid, which are a protein mixture chiefly, composed of type I collagen.

The length of the columnar region is determined by the rate of differentiation of periarticular into columnar chondrocytes, the rate of proliferation of columnar cells, and the rate of differentiation of columnar to hypertrophic chondrocytes. Hypertrophic chondrocytes express Indian hedgehog (IHH) [6] which is a member of hedgehog family of secreted signaling molecules [7]. Parathyroid hormone-related peptide (PTHrP) is expressed by periarticular chondrocytes [5]. PTHrP and IHH are important regulators of cartilage development [8]. IHH, produced by prehypertrophic and hypertrophic chondrocytes, stimulates production of PTHrP by periarticular chondrocytes [9,10]. IHH directly acts on periarticular chondrocytes to stimulate their
differentiation to columnar chondrocytes and also stimulates the synthesis of PTHrP by periarticular chondrocytes [8]. PTHrP, thereafter, binds to its receptor PTH/PTHrP receptor 1 (PTH1R), which is expressed at low levels by proliferating chondrocytes in columns and at higher levels by prehypertrophic chondrocytes [11] and prevents premature differentiation of chondrocytes into hypertrophic chondrocytes and thereby suppresses premature expression of IHH [8]. This negative feed-back loop exerted by IHH on PTHrP controls the synchrony of chondrocyte differentiation and regulates columnar chondrocyte mass [8,12,13].

The PTHrP also signals (through its receptor PTH1R) the core binding factor-α-1 which initiates the transcription of their downstream target MMP 13 [14-16]. The MMP 13 is strongly induced in hypertrophic chondrocytes, perioseal cells, and osteoblasts during endochondral ossification and intramembranous ossification [17-22]. It can degrade collagen types I, II, III and X [22,23]. The cartilage matrix composed of types II and X collagen is degraded and eventually calcified. This loss of collagen at the hypertrophic zone in the growth plate is associated with increased MMP 13 activity. MMP13 deficiency, therefore, would prevent orderly ECM degradation in developing growth plates with accumulation of types II and X collagens, and disturb endochondral ossification [4].

Even if MMP 13 is important for the replacement of cartilage with bone and not in the elongation of long bones, which may be controlled by its upstream signalling molecules. This explains the absence of MMP 13 mutation in HG syndrome patients and suggests a possibility of defect in the upstream signalling molecules. Although genome-wide linkage study is the ultimate tool when it comes to mapping disease loci, availability of families with multiple affected members is a severe limitation. Meanwhile in a disease like SEMD_{HG} with complex etiology, genes that could be postulated to be functionally related to the disease can be tested directly for their involvement in the disease process. Recent study [24] involving HG patients have identified defective hydroxylation of proline residues in patients and a possibility of defective vitamin D receptor [25]. Future studies are warranted to focus on the polymorphisms of the genes in concern.

Acknowledgments: The authors wish to acknowledge Ministry of Culture, Government of India for permitting to carry out this project under the national project, ‘DNA Polymorphism and Diseases’. We thank the social workers, Mr. Chandrashekar Bhat, Mrs. Rajakka and Dr. Najunda DC, Deputy Director cum Reader, CFSEIP, University of Mysore, Mysore in mobilizing the patients and their families. Authors are also thankful to Directorate of Health and Family Welfare Services, Government of Karnataka for providing their logistic support to this study. Above all, authors express their gratitude to the subjects who have willingly participated in providing blood samples.

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